

FIGURE 1



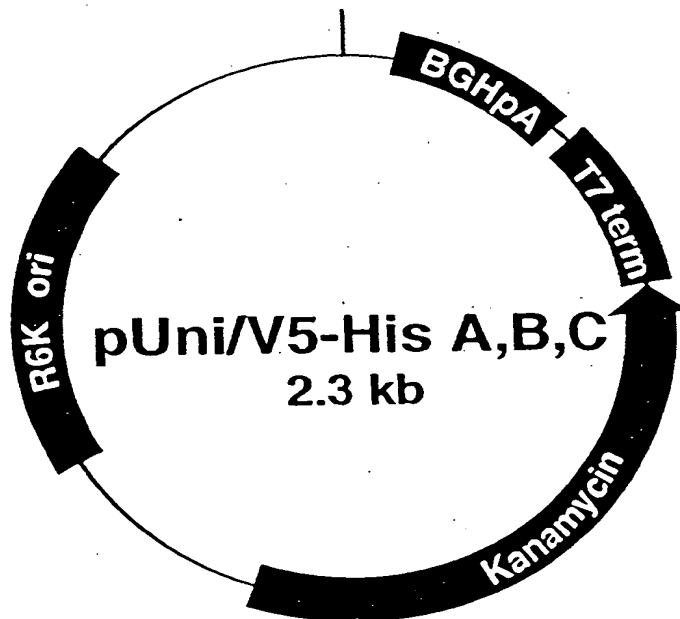
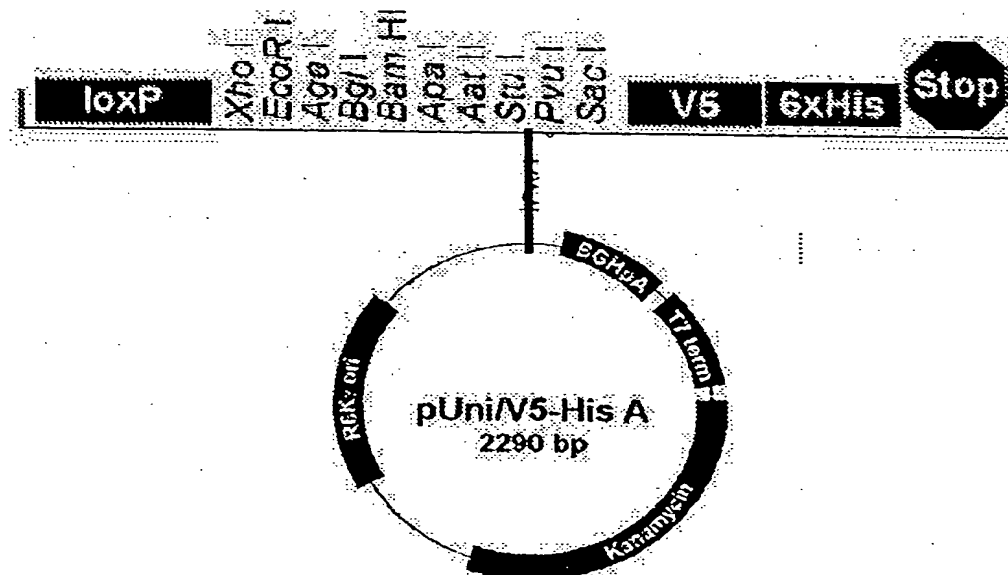
[illegible]

FIGURE 3

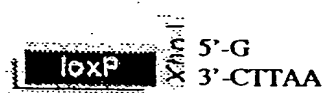
[illegible]

1. The first of these is the fact that the
 2. second is the fact that the
 3. third is the fact that the
 4. fourth is the fact that the
 5. fifth is the fact that the
 6. sixth is the fact that the
 7. seventh is the fact that the
 8. eighth is the fact that the
 9. ninth is the fact that the
 10. tenth is the fact that the

FIGURE 5



Add EcoRI and SacI  
digestion enzymes



resulting cohesive end  
post EcoRI digest



Resulting  
cohesive end post  
SacI digest

FIGURE 6

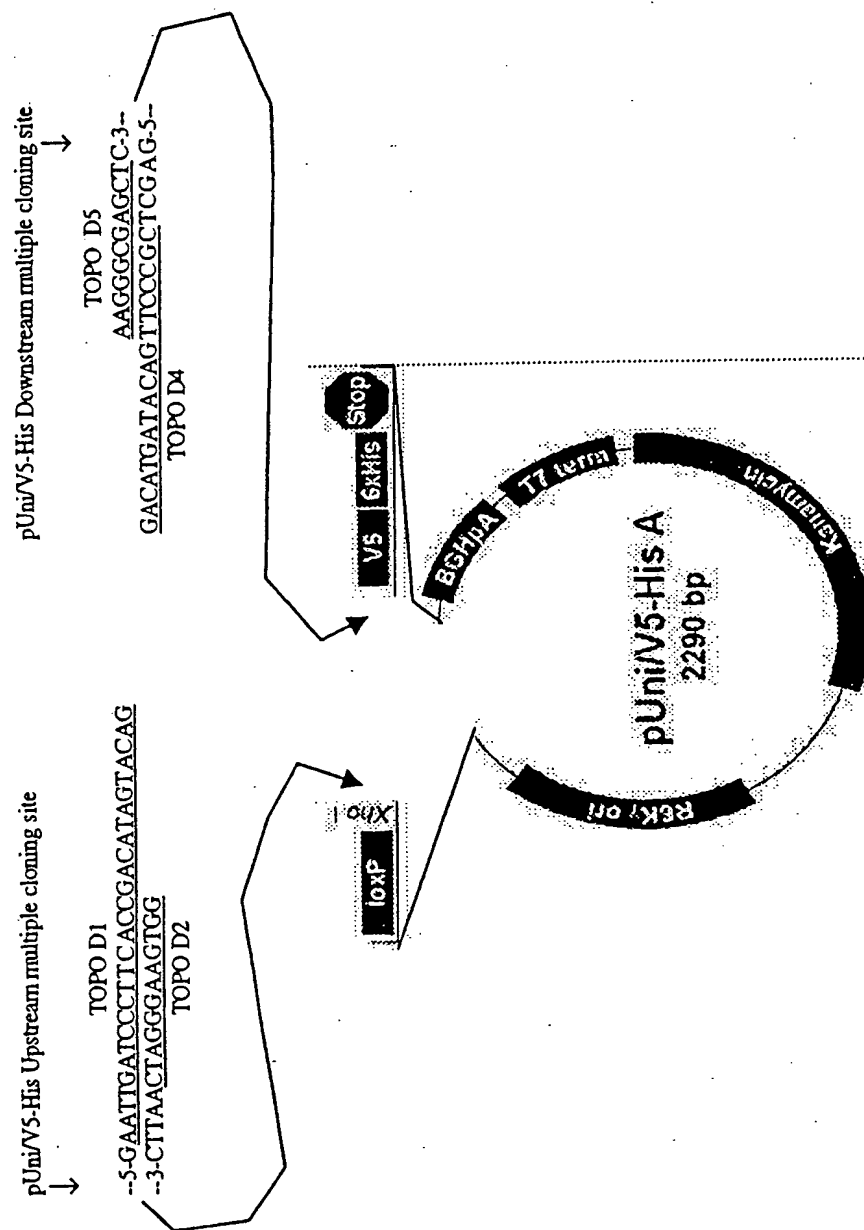


FIGURE 7

Diagram illustrating the ligation reaction:

Top strand: 5'-XXXXXXXXXXXXXXXXXXXXCCCTT  
 Bottom strand: 3'-XXXXXXXXXXXXXXXXXXXXGGA

Phosphate groups (P) are attached to the 3' end of the top strand and the 5' end of the bottom strand.

ADD TOPOISOMERASE

Bottom strand: 3'-XXXXXXXXXXXXXXXXXXXXGGA

Top strand: 5'-XXXXXXXXXXXXXXXXXXXXCCCTT

Phosphate groups (P) are attached to the 3' end of the top strand and the 5' end of the bottom strand.

TOPO (Topoisomerase) is attached to the 3' end of the top strand.

Leaving group is indicated by an arrow pointing away from the 3' end of the top strand.

FIGURE 8



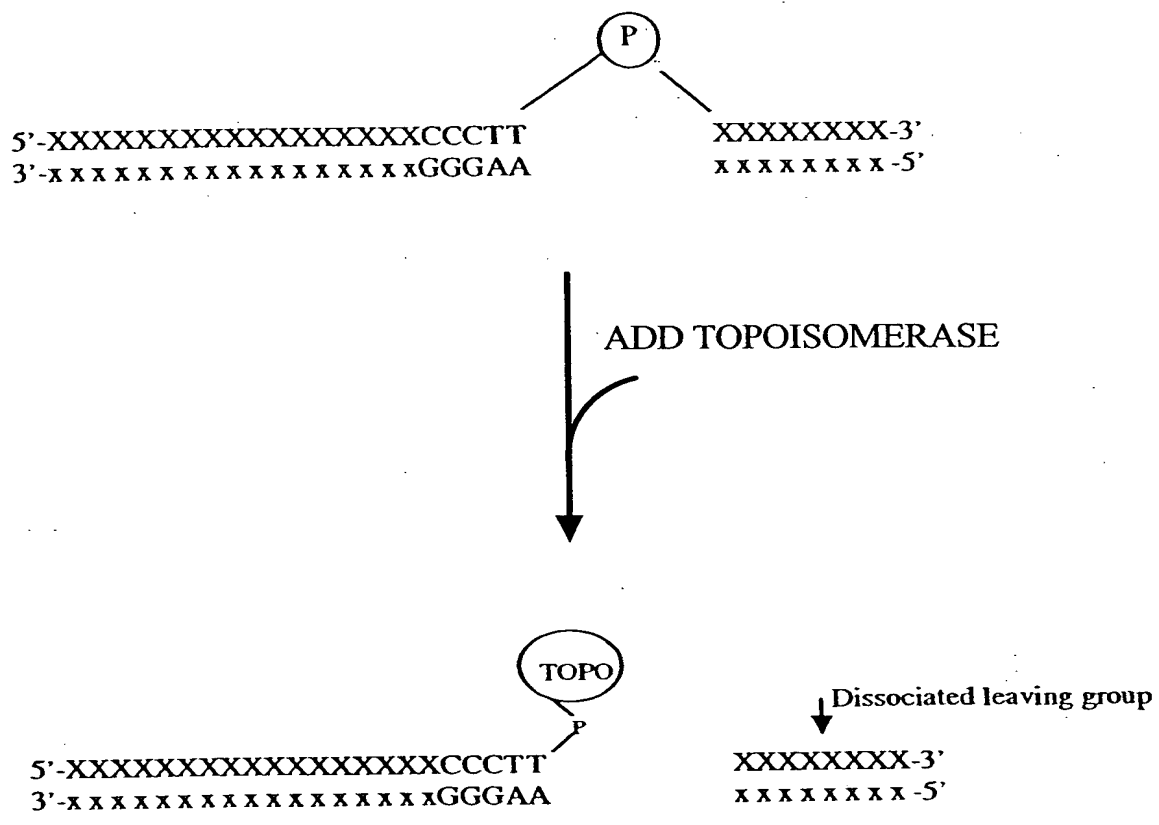


FIGURE 9

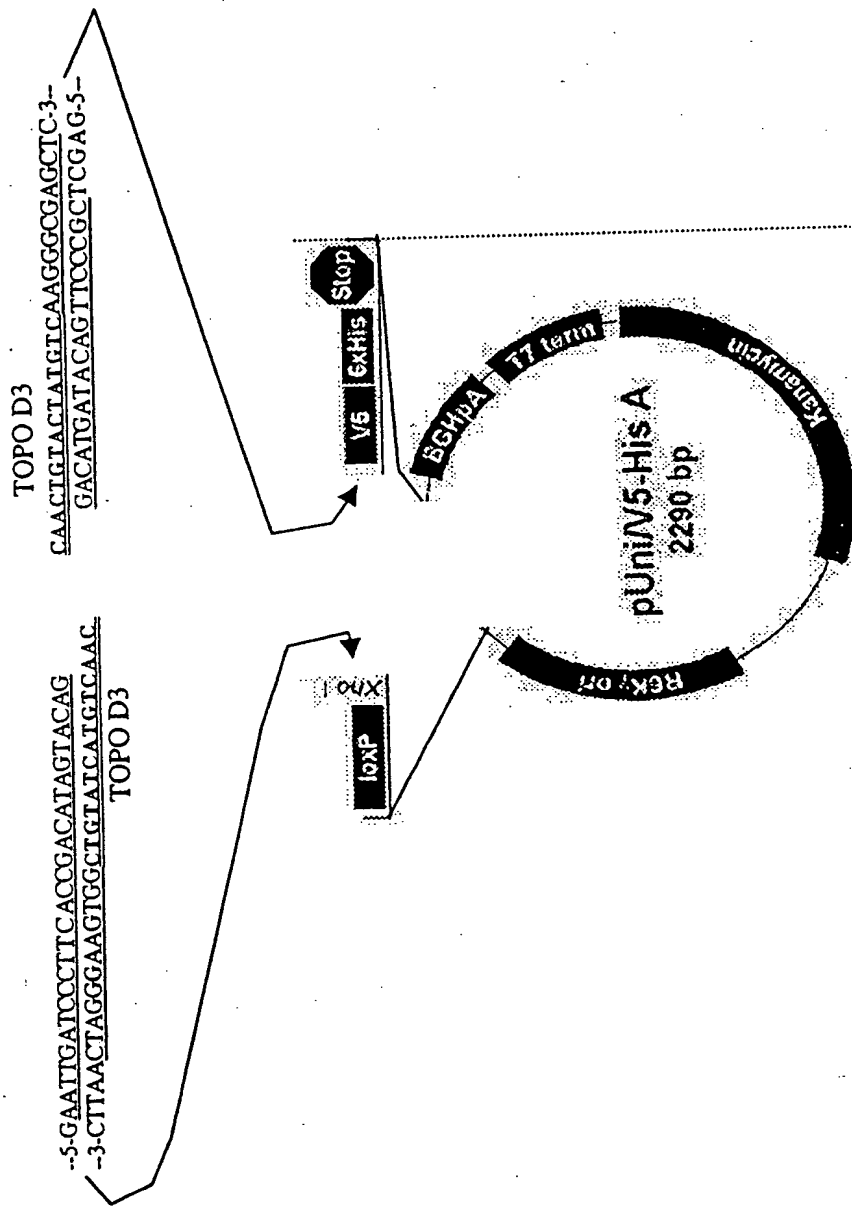


FIGURE 10

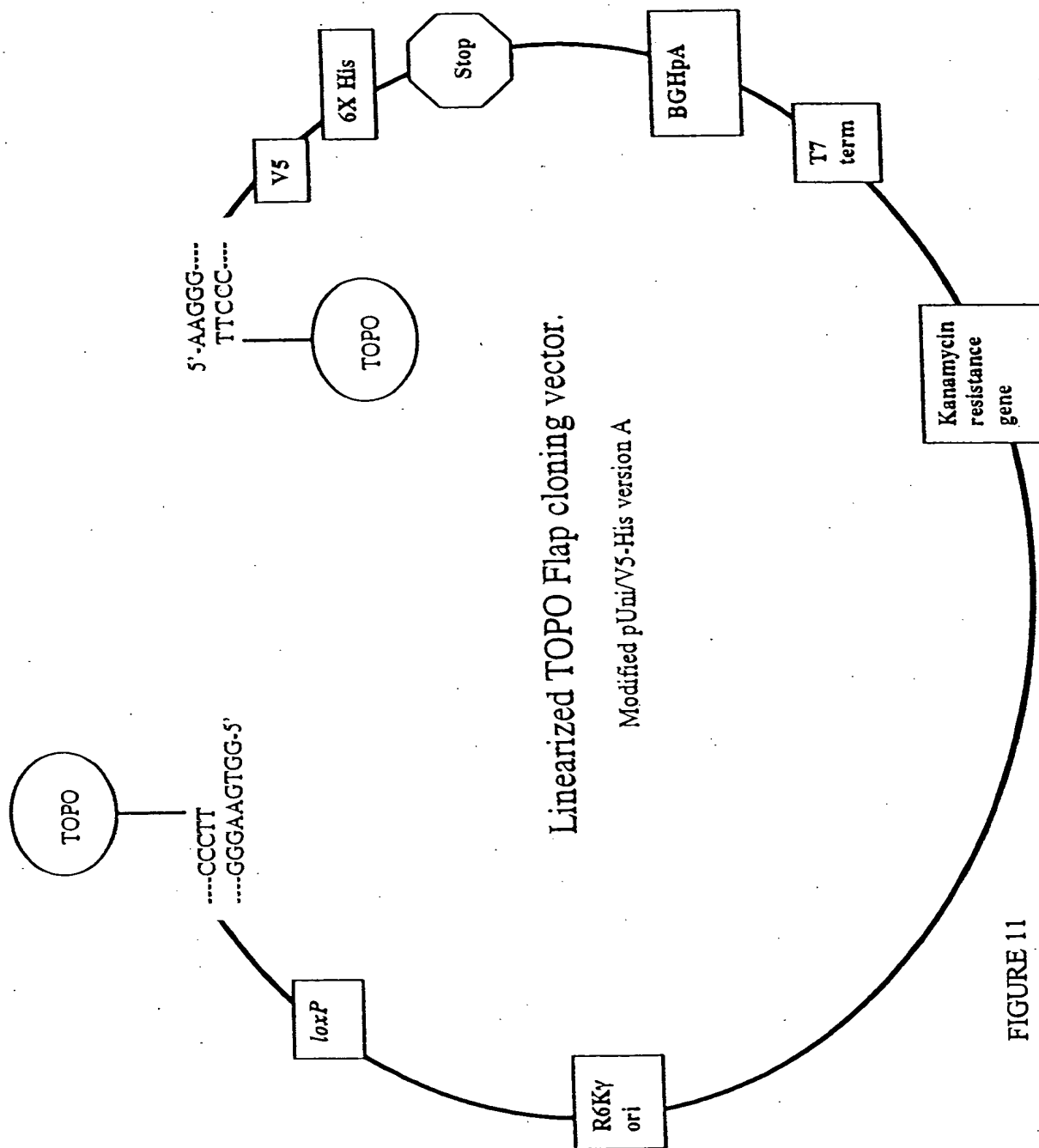


FIGURE 11

# Sequence of pCR 2.1

AGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATATGCAGCTGGCACGACAGGTT  
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAG  
GCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAA  
ACAGCTATGACCATGATTACGCCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCCGCAGTGTGC  
TGGAATTCCGGCTTAAGCCGAATTTGTCAGATATCCATCACACTGGCGGCCCGCTCGAGCATGCATCTAGAG  
GGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCAGTGGCCGTCGTTTTACAACGTCGTGACTGGGA  
AAACCCCTGGCGTTACCCAACCTAATCGCCTTGCAGCACATCCCCCTTTCCGCCAGCTGGCGTAATAGCGAA  
GAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCCGAGCCTGAATGGCGAATGGGACCGCCCTGTAGCG  
GCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCAGCGCCCTAGCGCC  
CGCTCCTTTCCGCTTTCTTCCCTTCCCTTCTCGCCACGTTCCGCCGCTTTCCCGCTCAAGCTCTAAATCGG  
GGGCTCCCTTTAGGGTTCCGATTTAGAGCTTTACGGCACCTCGACCGCAAAAACTTGATTTGGGTGATG  
GTTACAGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCCGCCCTTTGACGTTGGAGTCCACGTTCTTTAA  
TAGTGGACTCTTGTTCCAAACCTGGAACAACACTCAACCCCTATCGCGGTCTATCTTTTGATTATAAGGG  
ATTTTGCCGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTTAACAATTCAGGGCGCAAGGGCTGCTA  
AAGGAACCGGAACACGTAGAAAGCCAGTCCGCAGAAACGGTGTCTGACCCCGGATGAATGTCAGCTACTGG  
GCTATCTGGACAAGGGAAAACGCAAGCGCAAGAGAGAAAGCAGGTAGCTTGAGTGGGCTTACATGGCGAT  
AGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCAGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGT  
TGGGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGA  
TCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGG  
CCGCTTGGGTGGAGAGGCTATTCCGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT  
GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAA  
CTGCAGGACGAGGCGCGCGGCTATCGTGGCTGGCCACGACGGCGGCTTCCCTTGGCAGCTGTGCTCGACG  
TTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCG  
CCTTGCCTCCGCGGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCT  
ACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAAGCTGGATGGAAGCCGGTCTTG  
TCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACCTGTTCCGCCAGGCTCAAGGC  
GCGCATGCCCGACGGCGAGGATCTCGTCTGTATCCATGGCGATGCCTGCTTGGCGAATATCATGGTGGAA  
AATGGCCGCTTTCTGATTCAACAGCTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGT  
TGGATAACCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCCTCGTGTCTTACGGTAT  
CGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAATTGAAAAAGGA  
AGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTGGCGCATTTTGCTTCCCTGTTTT  
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CTTTTAAAGTTCTGCTATGTCATACACTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCCCG  
GGCGCGGTATTCTCAGAATGACTTGGTTGAGTACTACCAAGTACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAACACTTGGGCCAACTTACTTCTGACAA  
CGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGACAAACATGGGGGATCATGTAACCTCGCCTTGATCG  
TTGGGAACCGGAGCTGAATGAAGCCATACCAACGACGAGAGTGACACCACGATGCTGTAGCAATGCCA  
ACAACGTTGCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGA  
TGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATGCTGATAA  
ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAG  
GTGCCCTACTGATTAAAGCATTTGTAACCTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAA  
ACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAA  
CGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTT  
TTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCCGCTACCAGCGGTGGTTTGTGTTGCCGGATCA  
AGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATAACCAAACTACTGTCTTCTA  
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCAGCTTACATACCTCGCTCTGCTAATCC  
TGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGACTCAAGACGATAGTTACC  
GGATAAGGCGCAGCGGTCCGGCTGAACGGGGGTTCTGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC  
ACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACA  
GGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCAGGAGGGAGCTTCCAGGGGGAAACGCTGGTA  
TCTTTATAGTCTGTCCGGTTTCCGCCACCTCTGACTTGAGCGTCAATTTTTGTGATGCTCGTCAGGGGG  
CGGAGCCTATGGAAAAACGCCAGCAACGCGCCTTTTACGGTTCTGCGCTTTTGTGCTGGCCTTTTGTCTC  
ACATGTTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATAC  
CGCTCGCCGACCGGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAG

FIGURE 12

A

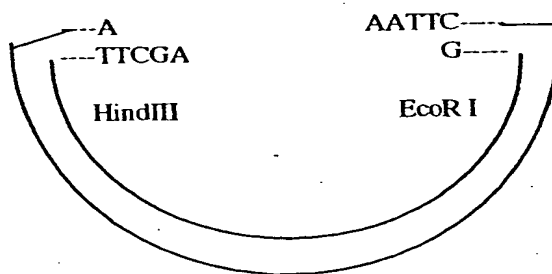
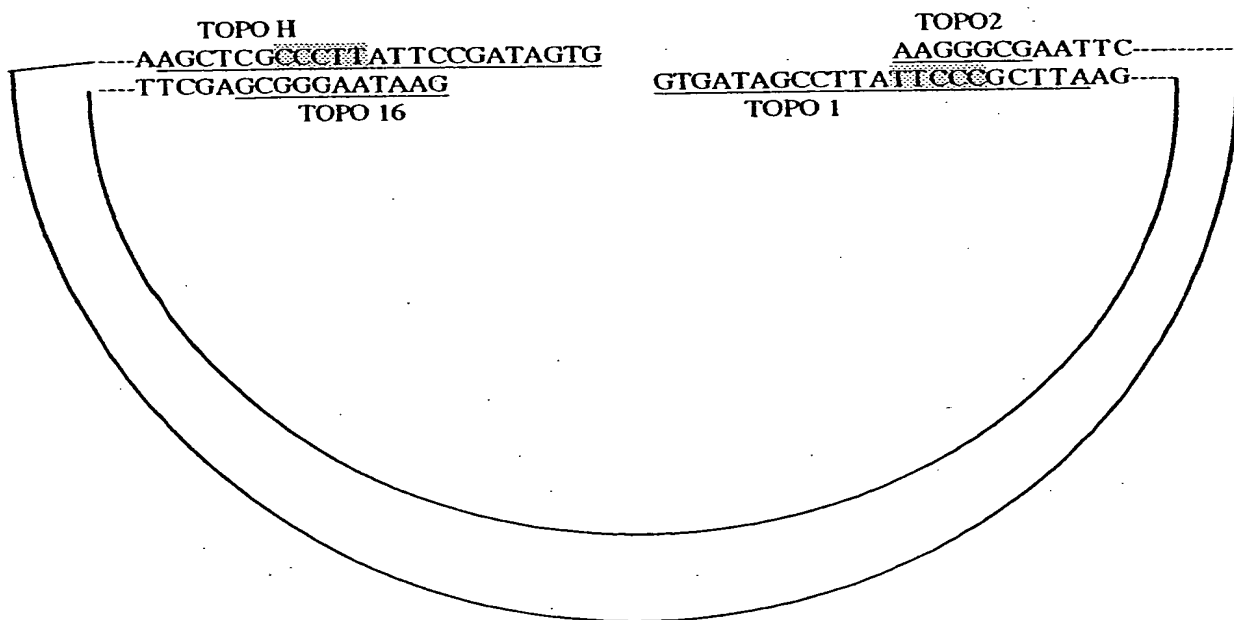
**B**

FIGURE 13



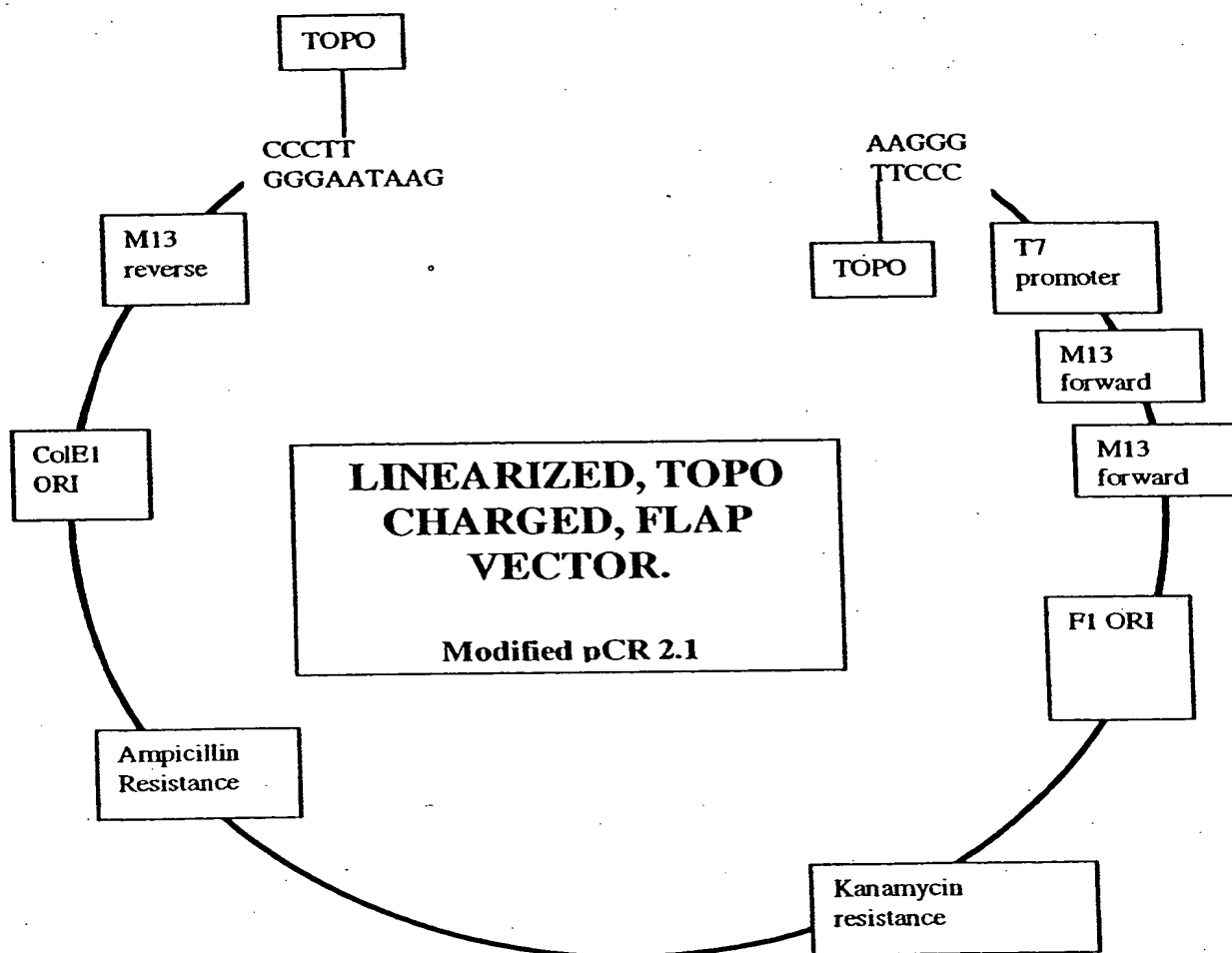
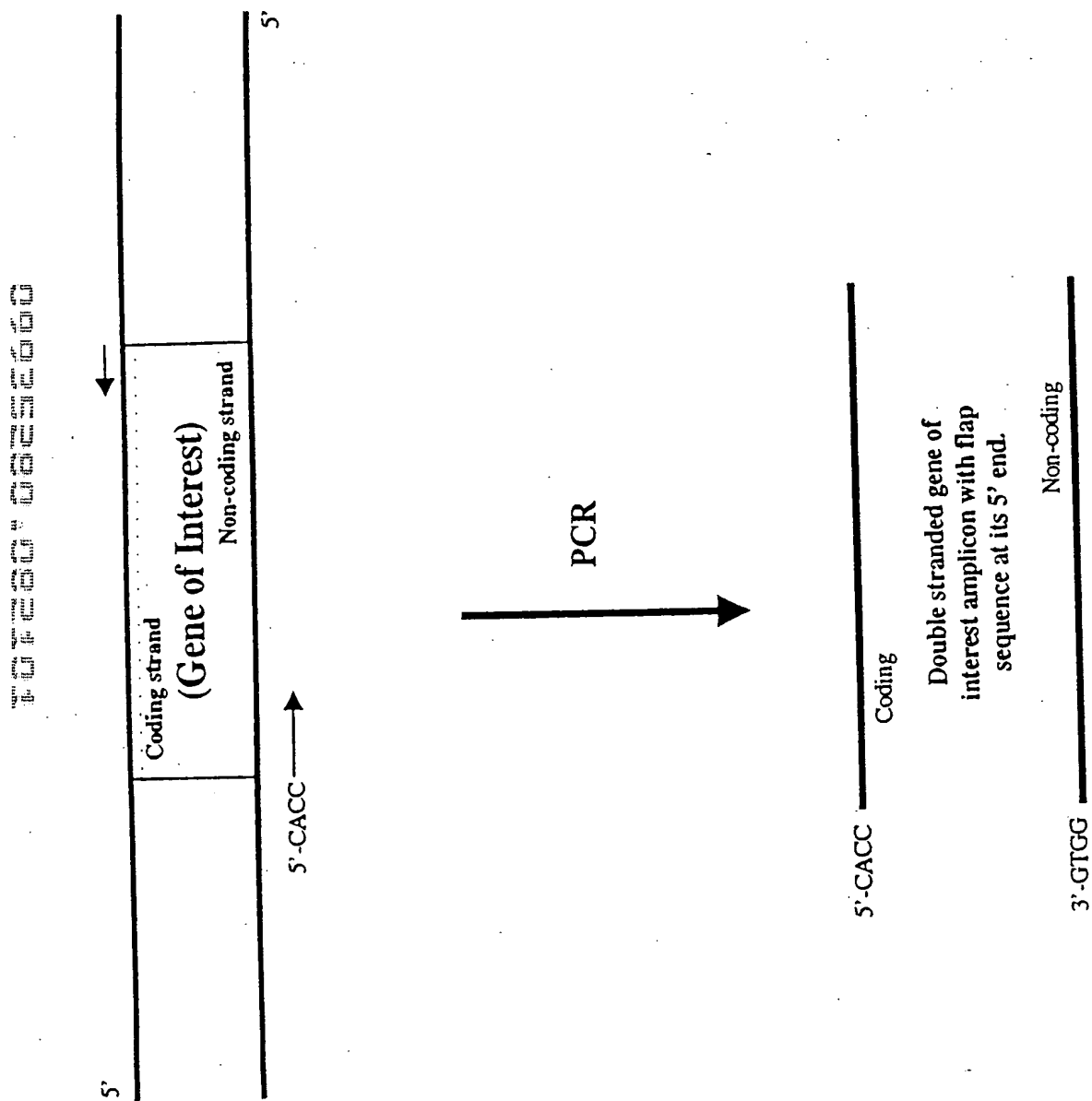


FIGURE 15





TOPO FLAP CLONING VECTOR

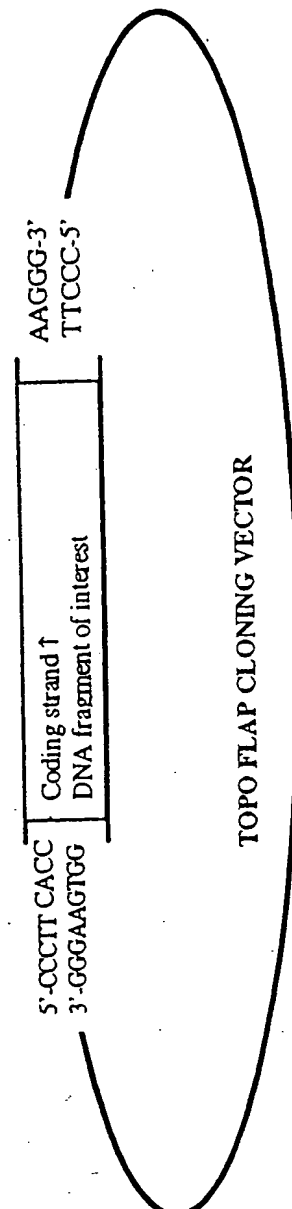
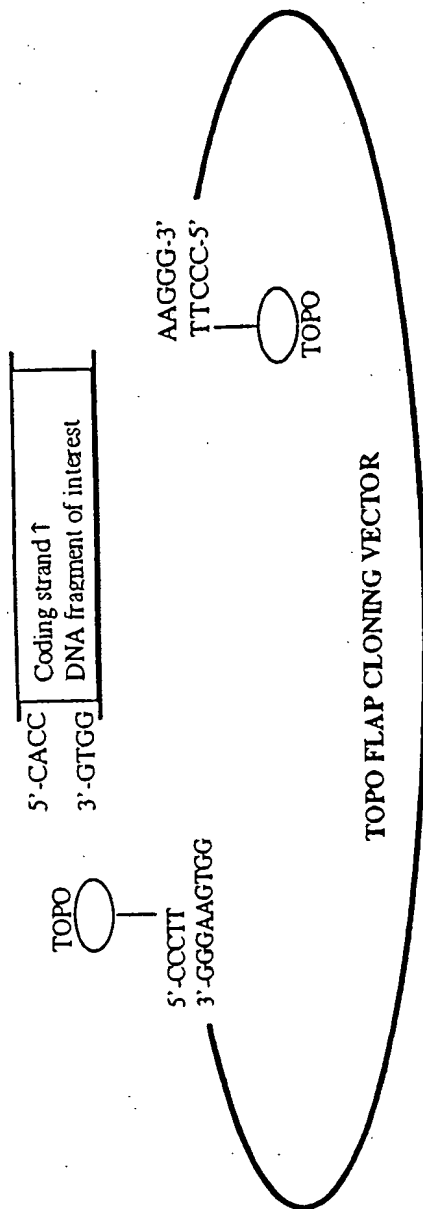


FIGURE 17